

REMARKS

1. General Matters

1.1. Species Restriction

The species restriction mailed July 29, 2003 was flawed in that the species, as defined, were not mutually exclusive as required by MPEP §806.04(f). For example, in the species restriction with respect to platforms, species 1 was peptides. However, peptides may be tandem repeats (species 3), linear (species 5), cyclic (species 6), fluorescent labeled (species 7), or lipidated (species 8). The same is true of the peptidomimetics of species 2 or the hybrids of species 3.

With regard to glycosylation sites, both natural (species 1), and unnatural sites (species 2) may be blocked or unblocked (species 3).

In view of the foregoing, we consider claims 23, 24, 25 and 28 to be generic to the elected platform (peptides).

1.2. We have amended Fig. 3, as recommended, to label the separate parts as "(a)" and "(b)", and made conforming amendments to the specification.

1.3. A substitute power of attorney was filed on May 12, 2004.

2. Claims

The present claims are directed to methods of making second and higher level glycopeptide libraries by steps which comprise random glycosylation of existing carbohydrate structures of a library comprising glycopeptides.

The "level" of a glycopeptide library indicates the number of rounds of glycosylation employed in synthesizing the library.

P9, L2-9 states

A first level library of a desired glycopeptide is created by primary glycosylation of the peptide with a single glycosyl donor or a mixture of donors.

Reaction of a core peptide with a glycosyl donor, or mixture of donors, results in a library of randomly glycosylated glycopeptides. A first level library... can form the basis for generating higher level libraries.

Thus, a first level library is the result of glycosylating unglycosylated peptides. A glycosyl donor can glycosylate such a peptide at any glycosylation site. For example, mono- and oligosaccharides can be N-linked to the peptide, by reaction with the NH₂ group on the side chain of Asn (or the NH₂-terminal of the peptide), or O-linked to the peptide, by reaction with the OH group on the side chain of Ser, Thr or hydroxylysine. P1, L21-28; P6, L14-20.

If the peptide were reacted with a single donor (i.e., random glycosylation were not employed), then the only way that a first level library could have the diversity implied by the term "library" would be if there were diversity in the amino acid sequence, e.g., by glycosylating a peptide library like that of P2, L9-30.

Random glycosylation is achieved by reacting the starting peptide(s) with a mixture of different glycosyl donors, such as galactosamine, N-acetylgalactosamine, and sialyl. P8, L9-21.

Claim 1, as amended, uses a first level glycopeptide library comprising glycopeptides which are diversely glycosylated, i.e., they vary in terms of which amino acids are glycosylated and/or what carbohydrate structures are attached to a particular AA glycosylation site. Such a first level library is conveniently obtained by random glycosylation of starting unglycosylated peptide(s), i.e., reaction of the latter with a mixture of glycosyl donors, cp. claim 44.

What, then, is a second or higher level library? According to P9, L10-25,

A second level library is created by

reacting one or more first level libraries with one or more further glycosyl donors. Prior to further reaction, unreacted glycosylation sites on the peptides may be blocked, e.g., by acetylation, in order to prevent these glycoforms from being eliminated from the library by being converted into different glycoforms. Following purification, the protecting groups of the carbohydrate structures on the glycoforms are selectively removed to create additional glycosylation sites on the existing carbohydrate structures. Random glycosylation with these additional donors further extends existing carbohydrate structures, thereby to create more complex glycopeptide structures. Higher level libraries are similarly created by reacting one or more second level or higher libraries with one or more further glycosyl donors.

When a glycosyl donor is reacted with a glycopeptide of a first level library, in theory it can react either (1) with a glycosylation site on the side chain of an unglycosylated amino acid, or (2) with a glycosylation site on a carbohydrate structure of the first level glycopeptide. If, prior to this second glycosylation reaction, the unreacted glycosylation sites on the amino acids are blocked, then possibility (1) is eliminated, and the second glycosylation can only extend the existing carbohydrate structure.

Amended claim 1 does not exclude possibility #1, but it does require that carbohydrate structure of at least some of the glycopeptides be randomly extended.

In reciting that peptides are "randomly glycosylated", applicants indicate that the starting peptide(s) are given the opportunity to react with the members of a mixture of glycosyl donors, and that some molecules are thus glycosylated. We do not mean to imply that every single peptide molecule must in fact be glycosylated; that would be contradictory to the teachings of P9,

L12-13 to the effect that some glycosylation sites may remain unreacted.

Claims 48 and 49, based on P11, L15-17, refer to the peptide as being derived from a "cancer-associated mucin" (48) or a "MUC1 core protein" (49).

We interpret "derived" to allow the peptide to be a fragment of the core protein of the mucin (e.g., MUC1), since there is specific disclosure of two fragments of MUC1 (a 16 a.a. fragment at P11, L17-21, and a four amino acid fragment in Example 1 on p. 13). We also interpret "derived" as allowing replacement of L-amino acids with the corresponding D-amino acids, in accordance with the teachings of P6, L21-25, labeling of amino acids with UV-active or fluorescent labels per P6, L26-28, cyclization of the peptide per P6, L26, and lipidation per P6, L37-P7, L2. Naturally, it also allows for glycosylation.

The four amino acid limitation is based on the GSTA disclosure in Example 1.

3. Prior Art Issues

3.1. Claims 1-2, 5-12, 14, 17, 19-20, 22 and 26-27 are rejected as anticipated by Vetter, et al. WO95/18971. Thus, by implication, the Examiner concedes that claims 3, 4, and 21 are not anticipated.

Claim 3 recites a 16-a.a. subsequence from the tandem repeat sequence of MUC-1. Claim 4 recites a tetrapeptide (GSTA) of that subsequence. Claim 21 recites that "said glycosylation sites consists entirely of d-optical configuration".

Vetter et al. (pp. 25-27) discloses the synthesis of glycoconjugate library of the form Ac-X-X-E(OAl)-X-P-resin, where Ac is acetyl, E is Glu, P is Pro, each X is randomly selected from a set of 18 side chain-protected AAs, and "OAl" is the allyl ester protecting group. First, a peptide library (diversity 18⁴) was synthesized. Then it was converted into a glycopeptide

library by removing the allyl ester and replacing it randomly with one of a set of 17 glycosylamines (P26, L29-31); these were mono- or disaccharides.

Thus, Vetter randomly glycosylated a glycosylation site on a "platform" (peptide) to create a first level library of glycosylated platforms, per step (a) of claim 1.

However, claim 1 as amended expressly recites further glycosylation of the first level library to create a second level library.

Scheme IV shows synthesis of the first level of a library, where Tn₁, Tn₂, Tn₃, TF₁, TF₂ or TF₃ is introduced at either of two glycosylation sites (R, R₁). Then Scheme V shows synthesis of a second level library by attachment of GlcNAc to the aforementioned Tn or TF to generate core 6₁, 6₂, 2₁, or 2₂. The key point here is that it appears that a second level library is intended to be one in which the new sugars are randomly attached to the CHOs of the first library. Such random extension of carbohydrate structure is mandatory in amended claim 1. No such step is disclosed by Vetter, so Vetter does not anticipate amended claim 1.

3.2. The same claims are also rejected as obvious over Schleyer et al. Schleyer taught producing a glycopeptide library by glycosylation of a "preferred peptide library". The main thrust of the Schleyer article is the use of a better support, the POEPOP resin, with one of three linkers. The linker is not a randomized element. Schleyer carried out glycosylation of the peptides with one of three sugars. However, it does not appear that the sugar was randomly selected. Thus, we do not agree with the Examiner that Schleyer teaches random variation of the sugar.

Even if we did, there is no teaching of "second level" variation which, as we have explained, is different from just glycosylating an unglycosylated peptide with two different glycosylation sites.

3.3. Claims 1-2, 5-14, 17, 19-22 and 26-27 are rejected as obvious over Rao, et al. Rao creates a glycopeptide library using fucose-serine building blocks. No amino acid other than serine is glycosylated, and no sugar other than fucose is disclosed. Thus, there is no randomness in Rao's glycosylation step. Rather, the randomness is in the amino acids to which the Fuc-Ser is attached.

The Examiner seeks to overcome this problem by reference to prior art cited by Rao, notably Peters et al. (1992), cited at col. 2, lines 55-60. The citation refers to use of protected Ser and Thr building blocks, but does not say what sugars were attached, and whether the sugars were randomized. (If the Examiner wishes to rely on Peters, it should be cited and made of record.)

To expedite prosecution, we have reviewed Peters, et al., J. Chem. Soc. Perkin Trans. 1:1163 (1992). This describes stepwise solid-phase synthesis of glycopeptides using already glycosylated amino acids as building blocks. Only one carbohydrate structure (GalNAc) was employed.

A multiple column peptide synthesizer was used for simultaneous assembly of forty different glycopeptides. These glycopeptide varied in amino acid sequence (see Fig. 1), and hence in the location of the carbohydrate structures, but the only carbohydrate structure employed was GalNAc. There was no randomization of the carbohydrate structures.

3.4. Claim 3 and 4 are rejected as obvious over Vetter or Rao or Schleyer in view of Ding. Ding discloses the utility of the MUC1 sequence recited in claim 3. However, the primary references do not render obvious the second round glycosylation step of claim 1, and this deficiency is not addressed by Ding.

With respect to claim 4, Ding does not single out the GSTA as significant. So this claim offers a further distinction.

4. Enablement Issues

4.1. The Examiner concedes that the specification is enabling for MUC1 as the platform, but not for peptides more generally. She says that no other platforms are disclosed and "there is nothing in the specification that discloses the kind, type of residues in the platform that can be randomized".

The specification clearly contemplates use of platforms other than MUC1. Peptides, and in particular the core proteins of cancer associated mucins, are of particular interest (P1, L9-13). There is no reason to believe that other peptides would be more difficult to randomly glycosylate than would MUC1. Many different natural glycopeptides are known in the art. Of course, the starting peptide must feature at least one glycosylation site.

Platforms are discussed further at P5, L30-P7, L11. The specification says that the "platform" can be a peptide and, if so, that it may be linear or cyclic. It is also clear that the peptide may be composed of l- or d-amino acids. Reference is made to hydrophobic amino acids at P6, L35-37, and to glycosylatable amino acids at P1, L21-28.

Several specific platforms other than MUC1 are disclosed. The first is Tn antigen. Since Tn antigen is GalNAc-O-serine, the platform is just Ser per se. TF antigen has the same platform (P7, L30-34).

Another platform of interest is the one shown in Fig. 4. This is a peptide with an unusual bridged structure. See also the peptoid ($-\text{CH}_2\text{CH}_2-$ linkage) of Fig. 5. We also mention OSM and CA27.29 at P11, L11-14.

The starting peptide need not be a single peptide. Combinatorial chemistry is discussed in a general way at P2, L19-32. By implication a combinatorial library of unglycosylated peptides, or selected peptides from such a library, could be randomly glycosylated in two or more rounds to obtain a second

or higher level glycopeptide library. Of course, if some of the library members lacked glycosylation sites, these members would not be glycosylated.

4.2. On page 5, the examiner also raises questions as to the site of glycosylation. But it is clearly taught that for a given platform (peptide), N-glycosylation can occur at the NH₂-side chain of Asn, and O-glycosylation at the HO-side chain of Ser, Thr, or hydrolysine. P1, L21-28; P6, L16-20. These are "natural glycosylation sites".

With regard to coverage of unnatural glycosylation sites (P5, L35), there is disclosure of hydroxy functions for O-glycosylation and carboxy or carboxamido functions for N-glycosylation, see P6, L14-16.

We also make disclosure of the number, kind and length of glycosylation sites. A single site (on an unglycosylated peptide) is, of course, just a single amino acid. There can be just one such site (P5, L33), but 2-5 are preferred (P5, L36-37). The sites may differ as to whether they are N-linkable or O-linkable, and also as to whether they are clustered (P6, L35), and whether they are flanked by hydrophobic AAs (P6, L35-7).

Peptide and carbohydrate blocking groups are well known in the art. There is some discussion of blocking groups at P7, L2-11. At P27, L2-5 we mention acetylation of serine and threonine. This was done to prevent second round glycosylation of post-first round unglycosylated serine and threonine, but plainly could have been done earlier. There is also reference to Fmoc protection of Ser and Thr at P2, L10-15.

Kunz, Angew. Chem. Int. Ed. Eng. 26:294-308 (1987), cited on page 8, lines 36-37, discloses a large number of protecting groups used in carbohydrate chemistry, including phosphonoethoxycarbonyl (Peoc), 2-halo ethyl, (9-fluorenyl) methoxycarbonyl (Fmoc), tert-butyl ester, 2-(2-pyridyl) ethoxy carbonyl (2-Pyoc), 2-(4-pyridyl) ethoxy carbonyl (4-Pyoc), allyl

ester and allyloxycarbonyl (Aloc). Garg, et al., Adv. Carbohydr. Chem. & Biochem. 50:227-310 (1994), cited at P8, L37-P9, L2, cites additional protecting groups, including 4-methoxybenzyl (Mpm), allyl, benzyl, heptyl, 4-nitrophenyl, tert-butyloxycarbonyl, 2-triphenyl phosphonethoxycarbonyl, benzyloxycarbonyl, pentachlorophenyl, pentafluorophenyl and phenylacetyl.

4.3. The Examiner also questions the adequacy of the disclosure of the number, kind and length of the carbohydrate. We generally disclose "carbohydrate structures". We also refer to those associated with bacterial adhesins (P7, L26) and malignant cell antigens (P7, L29). The specifically named glycosyl donors include galactosamine, N-acetylgalactosamine, and sialyl (P8, L9-12). These are all monosaccharides. We also mention galactose and N-acetylglucosamine at P12, L9-13. Use of a disaccharide (TF antigen) as a glycosyl donor is suggested by P13, L25.

P8, L6-8 says that Fig. 7 shows examples of CHO structures found on cancer mucins. The depicted mucins are Tn, TF, STn, F1 α , STF, core 3, Core 5, sialyl core 3 and sialyl core 5.

Kunz, Angew. Chem. Int. Ed. Eng. 26:294-308 (1987), cited at P8, L36-37, discloses several carbohydrate structures found in naturally occurring glycoproteins, including sialic acid (P. 294), col. 2), galactose (same), glucose (P. 295, col. 2), xylose (same), N-acetylglactosamine (same), N-acetylglucosamine (P. 296, col. 1).

Garg, et. al., Adv. Carbohydr. Chem. & Biochem., 50:227-310 (1994), cited at P8, L37-P9, L2, notes in table I that the following carbohydrate residues occur as proximal sugars in mammalian glycoproteins:

- 2-acetamido-2-deoxy-D-glucose
- 2-acetamido-2-deoxy-D-galactose
- D-xylose

D-mannose

D-galactose.

On page 280, Garg discloses synthesis of carbohydrates containing L-fucose (compound 19c) and chitobiose (compound 23).

4.4. The Examiner says (1) "the disclosure does not enable the method of combining the different libraries to produced [sic] different levels of libraries" and (2) "it is not apparent as to what constitute [sic] a level [of a] library". If the Examiner doesn't know what we mean by a level, how can the Examiner be sure that a "mixed level" or "higher level" library is not enabled?

A first level library is made by randomly glycosylating an unglycosylated peptide at one or more glycosylation sites. P3, L27-33. (It can include some unglycosylated peptides which did not react with the available glycosyl donors.) A second level library is made by glycosylating the already glycosylated peptides, P3, L33-P4, L3.

It is clear that in making the second level library, the second round glycosylation may be confined to glycosylation sites on the attached sugar, i.e., to CHO chain extension. See P9, L10-16. However it is not necessarily so limited. Nonetheless, to qualify as a second level library, at least some of the glycosylation must be of previously attached sugars. This is discussed more particularly at P11, L21-33.

That also means that you cannot necessarily tell by looking at an individual glycopeptide whether it is from a "second level library" or a first level one. If the glycopeptide has a disaccharide attached, it might have been the result (1) a single reaction of a peptide with a disaccharide donor, or (2) two successive reactions with monosaccharide donors. However, the present claims are method claims, and hence positively recite a second round of glycosylation.

4.5. The Examiner asserts that even for the disclosed tandem repeat, the different glycosylation sites and the variety of carbohydrates which can be introduced at those sites results in an "unimaginably large" number of possible glycopeptides.

The test of enablement for a method claim is not the number of products which the method can produce, but rather whether the amount of experimentation necessary to practice the method.

Combinatorial synthesis techniques, by their very nature, make it easy to generate a very large number of different variants. In the peptide library field, libraries with 10^8 - 10^{10} different sequences are routinely synthesized and screened. It would be easy to synthesize more diverse libraries, too. If 20 different amino acids are allowed at each variable position, the diversity of the library is 20^n , where n is the number of variable positions. Increasing " n " is trivial.

Here, the diversity of a strict one level glycopeptide library, synthesized by randomly reacting peptides with i glycosylation sites with j different monosaccharide reagents is j^i , if all sites are reacted. If this first level library is then reacted in a similar way (each added carbohydrate structure provides a single new glycosylation site, the same reagent mixture is used, and all sites are reacted), the diversity of the second level library is j^{2i} . Thus, with, SEQ ID NO:1, which has five glycosylation sites, use of four donors in each round results in a diversity of $4^{2 \cdot 5} = 4^{10} \sim 10^6$.

If we allow some glycosylation sites to go unreacted, then the diversity of the second level library is $(j+1)^{2i}$, or $5^{2 \cdot 5} = 5^{10} \sim 10^7$.

The difficulty of the synthesis is not increased significantly by adding increasing the number of glycosylation sites, the number of donors, or the number of rounds of glycosylation, because we are not trying to synthesize a specific glycopeptide.

5. Definiteness Issues

5A. We have amended claim 1 to avoid recitation of an optional step. However, we did this because we think optional steps are best recited in dependent claims, and not because we agree with the rejection.

The Examiner's statement "during synthesis the unreacted glycosylation sites have to be positively blocked" is wrong. We only have to block sites at which we don't want glycosylation. The optionally blocked sites on the glycosylated platforms (peptides) are sites which, despite opportunity, were not glycosylated during round 1. They are blocked so that in making a second round glycosylation, they are not glycosylated.

5B. The term "higher" in claim 2 meant higher in level than the last expressly recited level. Since that was fourth level, "higher" in claim 2 meant higher than fourth level, i.e., a library resulting from more than four rounds of glycosylation. Claim 2 has been rewritten for greater clarity; with this rewrite, "higher" is higher than second level.

5C. Claim 1 now recites glycosylation of a peptide (more precisely, a glycopeptide) rather than the broader term "platform". This moots the issue of antecedent basis for "peptide" in claims 3 and 4.

5D. The term "some" is not indefinite, it means more than one and less than all. The claim has been amended to make this explicit.

5E. We understand the Examiners concerns regarding the "unique" language (claim 17). However, if one thinks of the glycosylation site residues as X_2 , and the immediately adjacent flanking residues as X_1 and X_3 , then X_1 - X_2 - X_3 can be considered the site and its vicinity, and thus the requirement of claim 17 is that for each glycosylation site, the equivalent X_1 - X_2 - X_3 appears nowhere else in the peptide. For MUC1 (P11, L18), we would have VTS, TSA, DTR, GST, and STA, which indeed are all unique. We

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have rewritten claim 17 to make this explicit.

5F. A "cluster" (claim 27) of glycosylation sites is a series of consecutive glycosylation sites, i.e., a subsequence of two or more AAs in which all of the residues are selected independently from the group consisting of Asn, Ser, Thr, and hydroxylysine.

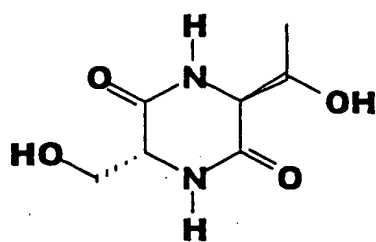
Respectfully submitted,

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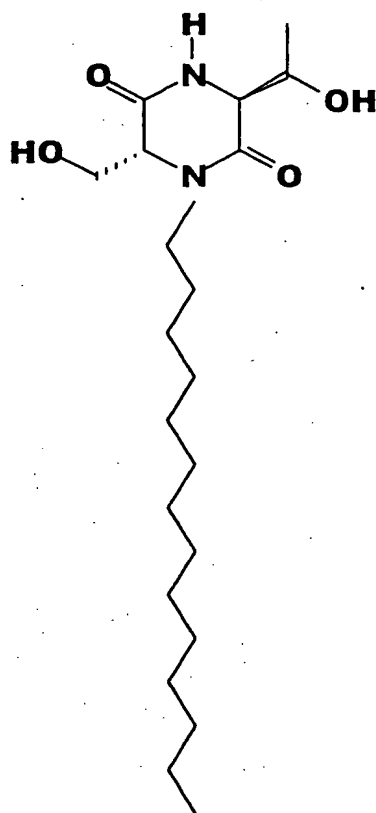
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(a) THE SIMPLEST CYCLIC PEPTIDE



(b) A SOLUBLE VERSION OF THE ABOVE (with C₁₄ lipid)

Figure 3